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Effects of the collembolan *Onychiurus subtenuis* on decomposition of *Populus tremuloides* leaf litter

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With 3 figures

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1. Introduction

Seastedt (1984), in an extensive review of the literature, concluded that although micro-arthropods make only a small direct contribution to soil metabolism their largest effect on decomposition is *via* their interactions with the microflora. Visser (1985) has classified these interactions into 3 main categories: (1) comminution, mixing, and channelling of litter and soil; (2) grazing on microflora; and (3) dispersal of microbial propagules. As McBrayer & Reichle (1971) have shown that 60% of the litter mesofauna are mycophagous, the second and third categories are likely to be most important for the majority of Collembola and mites.

While grazing can certainly alter the structure of fungal communities by changing the balance of interactions between competing species (Parkinson, Visser & Whittaker 1979 the net effects on overall decomposition rates are less clear. Van der Drift & Jansen (1977) observed increased O_2 uptake by fungal mycelia grazed by *Onychiurus quadriocellatus* Gisin, but Hanlon & Anderson (1979) found that *Folsomia candida* (Willem) could either stimulate at low densities, or inhibit at several higher densities, microbial respiration. In these microcosm experiments, the bacteria introduced into the cultures by the Collembola, thrived so that their standing crop increased with grazing pressure while that of the fungi decreased. Similarly, Visser, Whittaker & Parkinson (1981) attributed a significant increase in microbial respiration to bacteria and fungi tracked into microcosms by *Onychiurus subtenuis* (Folsom). Clearly these laboratory studies show that the dispersal of microbial propagules can potentially have an important influence on rates of organic matter decomposition.

Satchell (1974), however, sounded a cautionary note by pointing out that there was at that time, no evidence that the dispersal of common saprophytic micro-organisms limits decomposition rates in the field nor that it is affected significantly by soil invertebrates in nature.

The collembolan *Onychiurus subtenuis*, which as noted above has affected the structure and respiratory rates of very simplified microbial communities in laboratory microcosms, has also been observed in the field where it was found to undertake significant vertical migrations in response to summer rainfall in the cool temperate Aspen woodlands of Alberta (Hassall, Visser & Parkinson 1986). Introduction of specimens from the field litter layers onto sterile plates of malt extract agar has shown that they carry an average of 2.5 (for animals from the H layer) to 4.6 (for animals from the L-layer) spores per individual from a total of over 130 different taxa of fungi with a great deal of metabolic versatility (Visser, Hassall & Parkinson in preparation). Specimens from the lower horizons of the litter profile carried a range of fungal species not found on those from the top layers. One result of the rapid vertical migrations following remoistening of the surface litter could be that *O. subtenuis* might introduce inocula of different fungal species into the surface litter where they could potentially colonize patches of substrate exposed by grazing as they did in the laboratory

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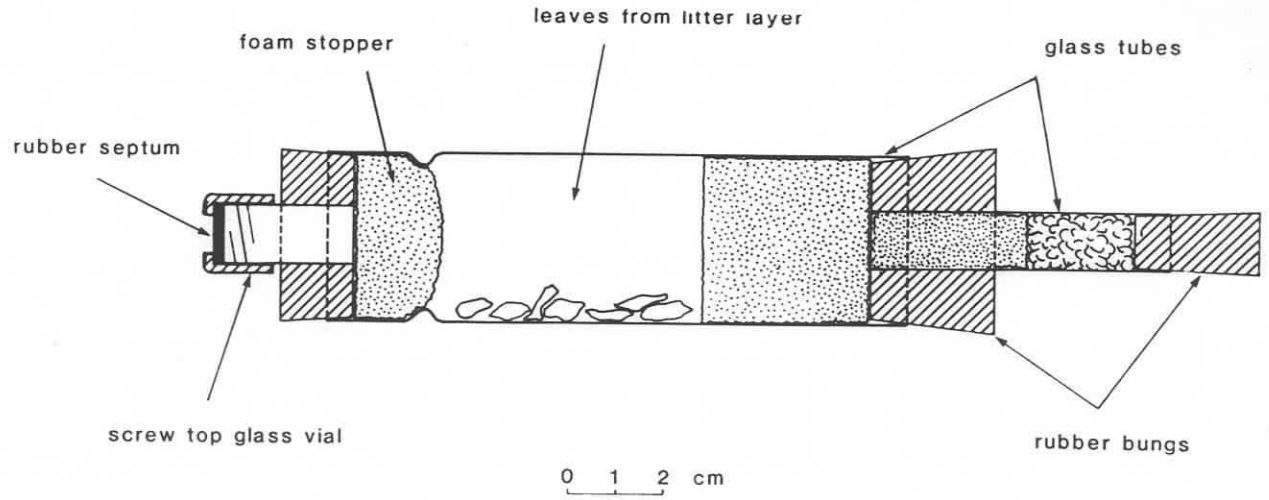

Fig. 1. Microcosm for studying microbial metabolism.

studies described by VISSER, WHITTAKER & PARKINSON (1981). This could then influence both the pattern and rate of microbial decomposition of the litter.

To test this hypothesis, microcosm experiments were designed on the basis of the field observations to represent, as realistically as possible, densities and the microbial species composition found in *Populus tremuloides* woodlands.

2. Materials and methods

Populus tremuloides leaves were collected from the surface of the L-layer on the Aspen woodland study site at Kananaskis described by VISSER & PARKINSON (1975), during early September (i.e. approximately 10 months after leaf fall). They were air dried in the laboratory and then stored at 5 °C until used when they were soaked in distilled water for 24 h. After surface water had been removed by blotting them between paper tissues, the petioles and midribs were removed and the laminae divided into six sections. One section from each of 42 leaves was placed in each of 6 microcosms so all contained the equivalent of 7 whole leaf laminae weighing in total approximately 0.5 g dry mass (dm) as 0.50 ± 0.095 g was the mean mass of L layer litter in cores of 23 cm² cross sectional area taken from this site during a study of the vertical migration of *Onychiurus subtenuis* (HASSALL, VISSER & PARKINSON 1986). In August samples an average of 9.9 *O. subtenuis* were found in the L and F layers of 23 cm² cores. In order to replicate field conditions as closely as possible, 10 *O. subtenuis* were, therefore, used with each 0.5 g sample of litter.

The Collembola were collected by live extraction in large 42 cm diameter Tullgren funnels from F_2 layer litter, into jars lined with moist plaster of Paris covered with a layer of moist F_2 litter in which the Collembola were kept for 2—3 days prior to setting up the experiments.

Two separate experiments were conducted with the leaf substrate in different conditions. In the first, the leaves were autoclaved to determine whether the propagules carried from the deeper layers were able to colonize L-layer leaves in the absence of other competing microorganisms. In the second, the leaves were simply remoistened in sterile distilled water and so had an extensive microbial flora already present and active when the Collembola were introduced. In both experiments microcosms into which no animals were introduced were used as controls.

In the first experiment, in order to distinguish between effects from migrating Collembola inoculating L layer litter with spores carried from the F_2 layer and their subsequent effects due to further dispersal of propagules and grazing, two treatments were used. In both, 10 *O. substenuis* were placed on the experimental leaves using sterilized mounted needles. In the first treatment all the animals were removed using sterile instruments after 24 h, to investigate the effect of inoculation alone, whereas in the second, the Collembola were left for the full duration of the experiment (28 days) to graze and further disperse the microflora on the leaves.

The microcosm consisted of a glass cylinder containing the leaves with foam stoppers at each end as shown in Fig. 1. To estimate rates of CO_2 output the microcosms were sealed at both ends with rubber bungs. At the inlet end (right of diagram) the bung contained a smaller glass tube packed with a foam stopper and cotton wool to filter spores, etc. from incoming air. At the outlet end, the rubber bung contained a screw topped vial with the bottom removed to make it into an open tube and a disc cut from the lid exposing a rubber septum. Before each experimental incubation each microcosm was flushed by pumping air, first saturated with water in a bubble jar, through it for 2 minutes.

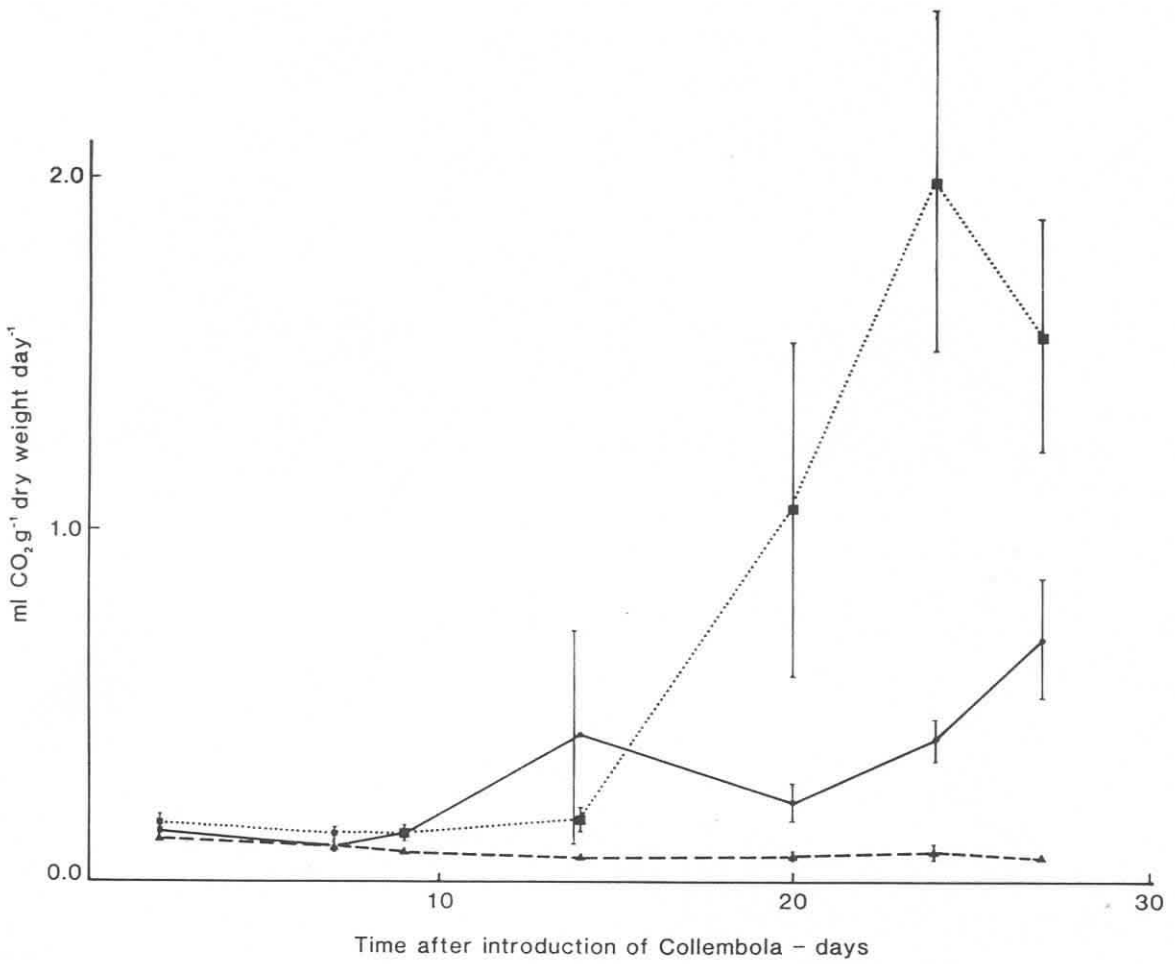

Fig. 2. Carbon dioxide production from autoclaved *Populus tremuloides* leaf litter; ▲— — —▲ without animals, ●————● 10 Collembola removed after 24 h, ■·····■ 10 Collembola present throughout the experiment.

After flushing, the septum lids were screwed into place and smaller bungs inserted into the inlet tubes so that the microcosm were completely sealed. They were then incubated in a controlled environment cabinet at 12.5 °C (the mean August air temperature at the study site) for approximately 24 h. The precise time for which they had been closed was noted and a 50 μl sample of the air within the microcosm was taken using a precision, gas-tight microsyringe with a 50 mm needle inserted through the rubber septum. The sample was then injected into a Fisher Model 1100 gas chromatograph and the CO_2 concentration calculated from the resulting chromatogram having calibrated the instrument with samples of known concentrations of CO_2, as described by MITCHELL (1973).

Concentrations of CO_2 were converted to rates by measuring the volumes of the microcosm and the moisture content of the samples so that results could be expressed in ml CO_2 g^{-1} d.m. day^{-1} after being corrected for the respiration of the Collembola as appropriate. The rate of CO_2 output from *O. subtenuis* was measured by enclosing specimens in small 1 ml screw topped vials on moist sterilized filter paper and incubating them at 12.5 °C then removing air samples with the gas tight syringe and analysing them with the gas chromatograph.

Between experimental incubations the large rubber bungs were removed to allow relatively free gas exchange. The microcosms were kept at 12.5 °C in 30 cm × 60 cm × 12 cm trays lined with 3 cm of foam saturated with water and covered with a perforated polythene top to maintain a high relative humidity, so preventing the leaf samples from drying out.

3. Results

The respiratory rates of micro-organisms on the autoclaved leaf litter are shown in Figure 2.

The sterile controls showed that no contaminants were getting into the microcosms through the foam stoppers as there was no detectable increase in concentration of CO_2 during incubation throughout the experiment. The treatment in which 10 *O. subtenuis* were introduced onto the leaves for 24 h then removed, started to show an increase in CO_2 production com-

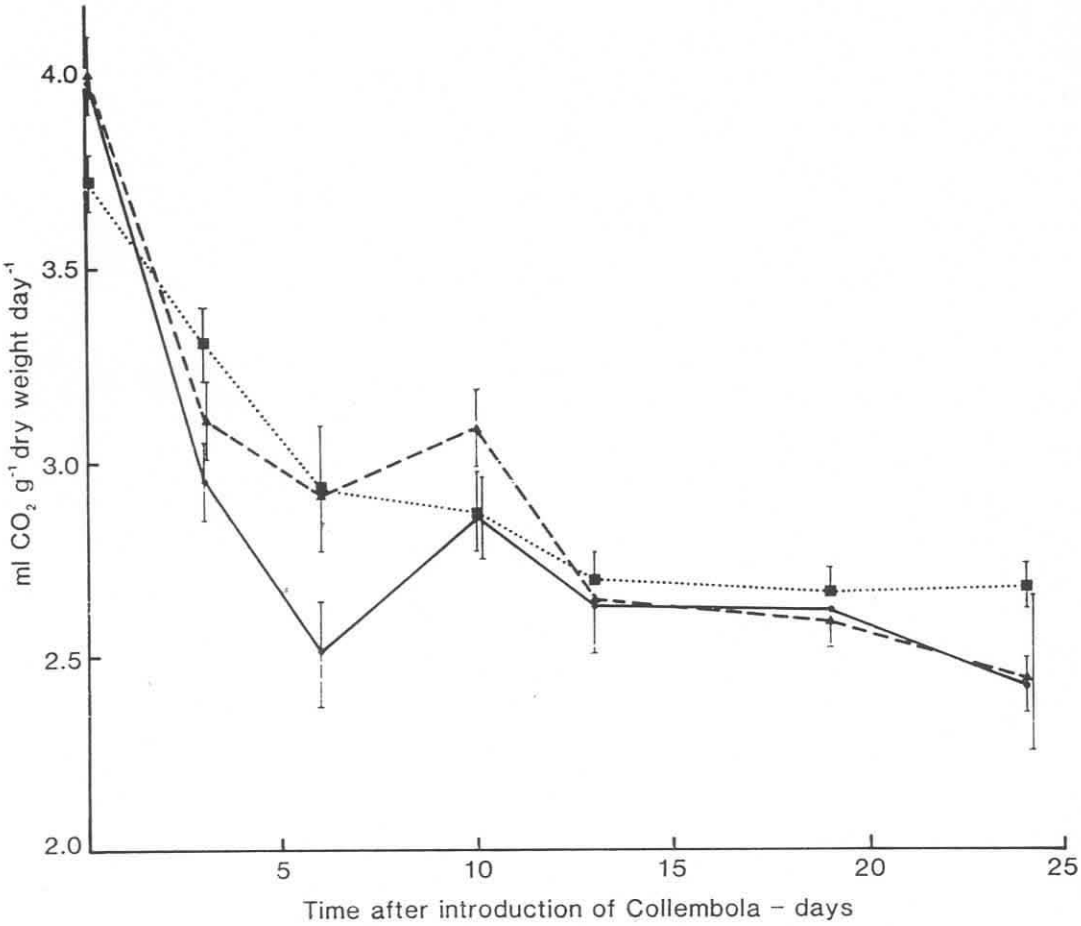

Fig. 3. Carbon dioxide production from remoistened *Populus tremuloides* leaf litter with normal microflora present; ▲— — —▲ without animals, ●————● 10 Collembola removed after 24 h, ■·····■ 10 Collembola present throughout experiment.

pared with the controls 7 days after the animals had been introduced and continued to show higher rates of CO_2 output for the rest of the experiment. Clearly the microbial propagules carried from the F_2 litter layer by *O. subtenuis* are capable of colonizing the L layer litter when introduced onto it in an autoclaved state in the absence of normal L layer microflora. The second experimental treatment in which 10 Collembola were introduced but then left with the leaves and developing microbial colonies throughout the experiment showed that a substantially greater rate of microbial metabolism resulted from their presence. This could have been due to the animals further dispersing mircobial propagules from the initial colonies to new areas of substrate and/or due to a stimulating effect of grazing on the developing microorganisms.

In the second experiment with litter that had simply been remoistened but not autoclaved, the pattern of microbial respiration appeared to be very different (Figure 3). The rate of CO_2 output from all three treatments started at double the peak production rate in the most vigorous of the three autoclaved treatments, indicating that there was already an active and vigorous microflora present on the leaves. The rate of CO_2 production in all three treatments declined during the first 10 days of the experiment as the stimulating effects of remoistening and disturbance decreased but the addition of the 10 animals for 24 h did not cause any increase in CO_2 production in this experiment. On the contrary, the levels for this treatment were slightly lower than for the control without animals for most of the experiment. Similarly, the treatment in which 10 Collembola were present throughout the experiment did not have significantly higher rates of CO_2 output than the treatment with no animals for most of the experiment, although CO_2 production did not decline as much during the 19—24 day period as it did in the other treatments.

4. Discussion

These results confirm the observations of VISSER, WHITTAKER & PARKINSON (1981) that *O. subtenuis* can inoculate leaf litter from the L layer of *Populus tremuloides* woodlands with micro-organisms that are metabolically capable of colonizing and decomposing this substrate. As VISSER, HASSALL & PARKINSON (in prep.) have shown that the propagules carried by Collembola from the F_2 layers are different from those most characteristic of the L layer, it seems possible that the vertical migration of Collembola observed following summer rains, by HASSALL, VISSER & PARKINSON (1986), could alter patterns of fungal succession by accelerating the rate at which leaves are colonized by fungal species characteristic of the lower F and H layers of the profile.

However, there is no evidence to suggest that the dispersal of fungal propagules from lower in the profile has any effect in enhancing rates of litter decomposition when the leaves are already complete with their natural flora of L-layer micro-organisms. It would appear that in the competitive milieu of the litter all the readily available metabolic substances are fully exploited by the wide range of microbial species found there and that if, as a result of the grazing activities of the Collembola, some patches of substrate are exposed in a way that promotes colonization by the propagules carried into this litter by the animals, then the rates of subsequent decomposition do not differ significantly from the rates at which the normal litter microflora would decompose the same substrate.

At first these results do not appear to be compatible with those of ADDISON & PARKINSON (1978) who observed that when *Folsomia regularis* was added to cores of arctic soils the microbial respiratory rate was significantly increased. However, these cores had been sterilized before being reinoculated with microbes so it is possible that some stimulation could have resulted from the animals moving propagules to otherwise uncolonized substrate in a similar way to that in which the *O. subtenuis* left on autoclaved leaves throughout the first experiment dispersed micro-organisms onto fresh substrate and so greatly enhanced microbial respiration.

However, Collembola very rarely encounter sterilized and uncolonized substrate in their field environment where lack of nutrients is considered to be a major factor contributing to the general biostasis of the soil/litter system (SATCHELL 1974). Within such a competitive environment, more realistically represented by the remoistened non-sterilized litter treatments, the addition of some extra propagules by the Collembola is unlikely to accelerate rates of litter decomposition unless the introduced inocula are capable of utilizing a completely different biochemical component of the litter substrate. Even though the spores carried by *O. subtenuis* from the F_2 layer are from species not regularly found in the surface litter, the results presented in Figure 3 show that when competing against the established litter microflora their presence does not significantly enhance the rate at which the litter is decomposed.

When lack of nutrients is the most important factor limiting microbial activity, grazing by mycophagous animals could be expected to mobilize some nutrients that would otherwise remain unavailable in microbial biomass. If the fauna grazed selectively on senescent colonies, the net effect of reducing standing crop but releasing nutrients might be expected to stimulate decomposition rates as observed by VAN DER DRIFT & JANSEN (1977). If, on the other hand, the animals chose to ingest the most vigorously growing hyphae, as the experiments described by VISSER & WHITTAKER (1977) show to be the case with *O. subtenuis*, then removing actively metabolizing fungal biomass is more likely to result in inhibition of microbial metabolism as observed by HANLON & ANDERSON (1979) with the higher densities of Collembola in their microcosms. In this study, the densities were based on those observed in the field and were lower ($\equiv$2 on 0.1 g dm leaf) than the lowest used in HANLON & ANDERSON's experiments. Possibly because of their preference for actively growing hyphae, grazing by the Collembola in these experiments, as in those described by ANDREN (1984) using reinoculated barley straw and *Folsomia fimetaria*, had no stimulatory effects at all on the rates of microbial metabolism.

It can be concluded, therefore, that although more than 1,000 *O. subtenuis* m^{-2} move rapidly into the L layer of this *P. tremuloides* woodland whenever it is moistened by summer rains in order to graze on the micro-organisms there, and despite their carrying over 3,800 spores m^{-2} into it from over 100 species of fungi characteristic of the lower litter strata, the overall results of their inoculating, dispersing, and grazing activities do **not** have any significant effects on the rates at which this leaf litter is decomposed.

5. Acknowledgements

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HASSALL, M., D. PARKINSON & S. VISSER, 1986. Effects of the collembolan *Onychiurus subtenuis* on microbial decomposition of *Populus tremuloides* leaf litter. Pedobiologia **29**, 219—225.

To investigate the effects that *Onychiurus subtenuis* has on rates of litter decomposition when it migrates up into the surface litter layers carrying spores from F-layer fungi, laboratory microcosms were designed to monitor microbial metabolism when leaves were incubated with and without Collembola. When 0.5 g of air-dried leaves were remoistened to 70% water content (as in the field after rainstorms), autoclaved, and 10 Collembola from the F_2-layer introduced for only 24 hours, significantly higher levels of carbon dioxide were recorded over the following four weeks than for the control sterile leaves. When the Collembola were left on the leaves throughout the four weeks so that further dispersal of propagules and also grazing could occur, significantly greater rates of CO_2 output were observed. These experiments showed that spores carried by the animals could colonize and metabolize the L-layer litter.

The experiment was repeated using air-dried litter remoistened to 70% water content but **not** autoclaved so that a diverse microbial community was retained. Under these conditions neither the initial innoculation nor subsequent dispersal and grazing of the microorganisms by the Collembola accelerated rates of carbon metabolism.

It was concluded that the rapid migrations of *O. subtenuis* from the H- and F- to the L-layers do promote spore dispersal but that, under the conditions of high microbial competition existing in the L-layer material, the effects of this in stimulating overall decomposition rates are negligible.

Key words: soil fauna-microflora interactions, fungi, decomposition, Collembola, dispersal, grazing.

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